Patent claims.

1. A method for the production of a lysate used for cell-free protein biosynthesis, comprising the following steps:

a) a genomic sequence in an organism, which codes for an essential translation product that reduces the yield of cell-free protein biosynthesis, is replaced by a foreign DNA located under a suitable regulatory element, said foreign DNA coding for the essential translation product that additionally contains a marker sequence;

b) the transformed organism according to step a) is cultivated;

c) the organisms from the culture obtained in step b) are lysed; and

d) the essential translation product is separated from the lysate obtained in step c) by means of a separation process that is selective for the marker sequence.

2. A method according to claim 1, characterized by that the essential translation product is selected from the group consisting of "termination factors or proteins interacting with termination factors - in particular RF1, RF2, RF3,

eRF, L11 or HemK -, initiation factors or proteins interacting with initiation factors, elongation factors or proteins interacting with elongation factors, aminoacyl tRNA synthetases - in particular cysteinyl tRNA or tryptophanyl tRNA synthetase -, enzymes of the amino acid metabolism - in particular amino acid transferases, isomerases, synthetases -, phosphatases, nucleases, proteases, kinases, racemases, isomerases, polymerases and combinations of the above substances".

3. A method according to one of claims 1 or 2, characterized by that the marker sequence is selected from the group "streptag II, polyhistidine, FLAG, polyarginine, polyaspartate, polyglutamine, polyphenylalanine, polycysteine, Myc, gluthathione S-transferase, protein A, maltose-binding protein, galactose-binding protein, chloramphenicol acetyl transferase, protein G, calmodulin, calmodulin-binding peptide, HAT (= natural histidine affinity tag), SBP (= streptavidin-binding peptide), chitin-binding domain, thioredoxin, β-galactosidase, S-peptide (residues 1-20 of the Rnase A), avidin, streptavidin, streptag-I, dihydrofolate reductase, lac repressor, cyclomaltodextrin glucanotransferase, cellulose-binding domain, btag, nanotag".

4. A method according to one of claims 1 to 3, wherein the marker sequence and the chromosomal gene are expressed as a fusion protein, and

wherein the translated marker sequence does not affect the activity of the essential translation product in the organism.

5. A method according to one of claims 1 to 4, wherein the separation step is an affinity chromatography or an antibody assay.

6. A method according to one of claims 1 to 5, characterized by that the organism is a prokaryote or an eurokaryote, in particular selected from the group comprising "enterobacteriales (e.g. escherichia spec., E. coli), lactobacillales (e.g. lactococcus spec., streptococcus spec.), actinomycetales (e.g. streptomyces spec., corynebacterium spec.), pseudomonas spec., caulobacter spec., clostridium spec., bacillus spec., thermotoga spec., micrococcus spec., thermus spec.".

7. A lysate for the cell-free protein biosynthesis obtainable by a method according to one of claims 1 to 6, wherein the lysate has a reduced activity of an essential translation product.

8. A lysate for the cell-free protein biosynthesis according to claim 7, wherein the lysate has a reduced activity of one or several essential translation products selected from the

group "termination factors or proteins interacting with termination factors - in particular RF1, RF2, RF3, eRF, L11 or HemK -, initiation factors or proteins interacting with initiation factors, elongation factors or proteins interacting with elongation factors, aminoacyl tRNA synthetases - in particular cysteinyl tRNA or tryptophanyl tRNA synthetase -, enzymes of the amino acid metabolism - in particular amino acid transferases, isomerases, synthetases -, phosphatases, nucleases, proteases, kinases, racemases, isomerases, polymerases and combinations of the above substances".

9. The use of a lysate according to claim 7 or 8 for the cell-free protein biosynthesis.

10. The use according to claim 9, wherein by means of amber suppressor tRNA's natural and/or non-natural amino acids, in particular biotinyllysine, fluorescent amino acids and/or phenylanaline, are incorporated.

11. An isolated microorganism or an isolated cell, wherein a genomic sequence, which codes for an essential translation product that reduces the yield of cell-free protein biosynthesis is replaced by a foreign DNA located under a suitable regulatory element, said foreign DNA coding for the essential translation product that additionally contains a marker sequence.

12. A microorganism, as deposited under DSM 15756.

Legend of the figures.

Fig. 1

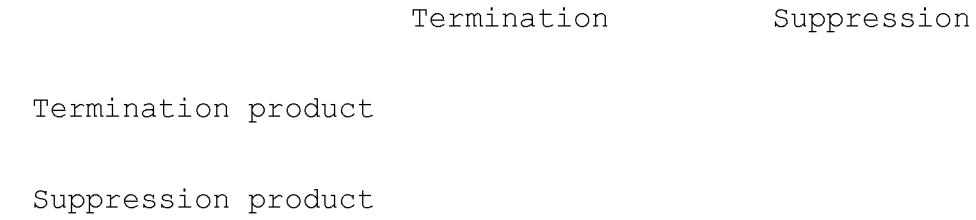


Fig. 2

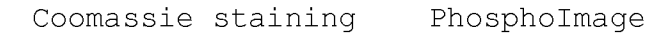


Fig. 3

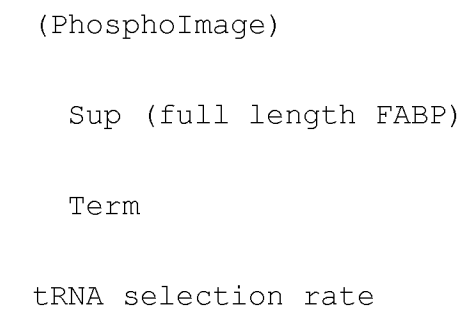


Fig. 4

A (PhosphoImage)

Suppression product

Termination product

B Protein synthesis E-PCR product

[arbitrary units]

C tRNA selection rate Synthesis at

termination factor

[arbitrary units]

Fig. 5

A + 400 mM NaCl (as preincubation)

without NaCl

Lysate - Run 1 - Run 2 - Run 3 - Wash fr. 1 - Wash fr. 2 - Wash fr. 3 - Wash fr. 4 - Wash fr. 5

B

Lysate - Run 1 - Run 2 - Run 3 - Wash fr. 1 - Wash fr. 2 - Wash fr. 3 - Wash fr. 4 - Wash fr. 5 - Elution fr. 1 - Elution fr. 2 - Elution fr. 3 - Elution fr. 4 - Elution fr. 5 - Elution fr. 6

C1

Lysate - Run 1 - Run 2 - Run 3 - Wash fr. 1 - Wash fr. 2 - Wash fr. 3 - Wash fr. 4 - Wash fr. 5

C2

Lysate - Run 1 - Run 2 - Run 3 - Wash fr. 1 - Wash fr. 2

Fig. 6

New

regulatory elements

Original regulatory elements

Fig. 7

A) Coomassie stain

B) Western blot

Fig. 8

Clone a

Clone b

Fig. 9

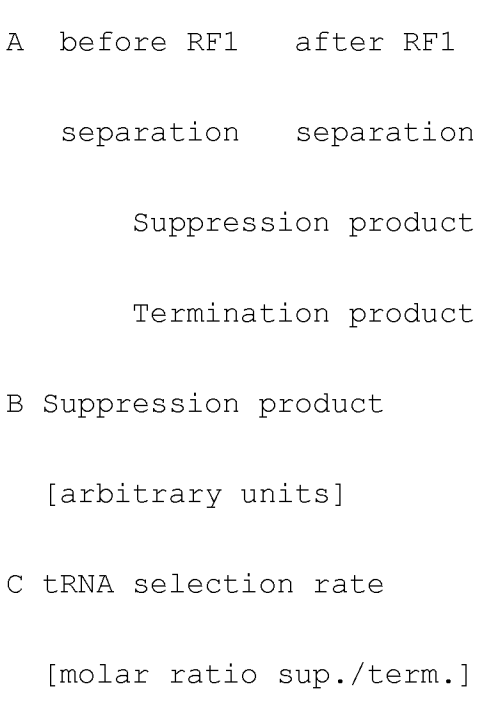
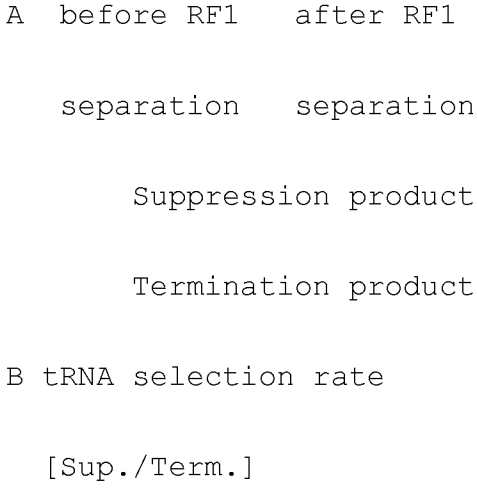

A before RF1 after RF1
separation separation
Suppression product
Termination product
B Suppression product
[arbitrary units]
C tRNA selection rate
[molar ratio sup./term.]

Fig. 10


A before RF1 after RF1
separation separation
Suppression product
Termination product
B tRNA selection rate
[Sup./Term.]

C Synthesis

[radioactive-marked protein]

Suppression product

Termination product

before RF1 after RF1

separation separation

Biotinyl tRNA [µM]

Fig. 11

A before RF1 after RF1

separation separation

BCCP

Biotinylated FABP

Biotinyl tRNA [µM]

B Suppression product

(biotinylated FABP)

[luminescence counts]

before RF1 after RF1

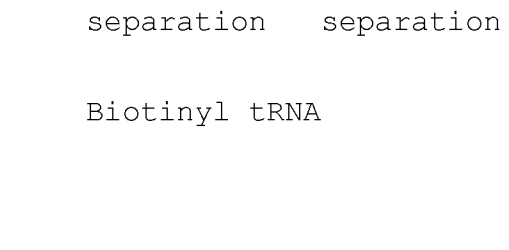
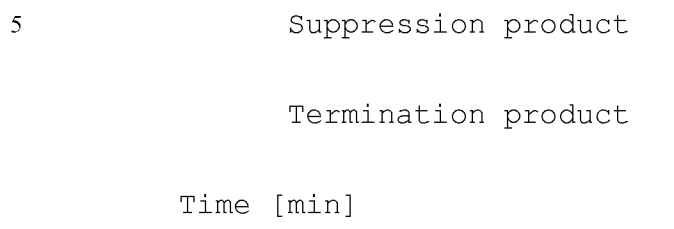

separation
separation
Biotinyl tRNA

Fig. 12


Suppression product
Termination product
Time [min]